REMARKS

Claims 1-26 are indicated to be pending in the present application, however, claims 16-17 have been canceled in a previous amendment, filed February 14, 2002. In the present amendment, claims 2-4, 6-9 and 26 are canceled, claims 1, 5, 10-12, 15, 18-19 and 25 are amended and new claim 27 is added.

The Office Action provides a Response to Arguments at page 2, line 10 through page 7, line 22 which indicates rejections which are maintained. As these remarks relate to the same rejections which appear to be repeated later in the Action at sections 6-30, Applicants refer the examiner to the sections below relating to each individual rejection for a response to these comments.

In Sections 4-5, the Action mentions the phrases "accumulated levels of p53" and "mutation load" in claim 1 and their definitions. Applicants have amended claim 1 to avoid these phrases. Applicants refer the Office to paragraphs 7 and 19 for support for these amendments. These paragraphs discuss how p53 protein is accumulated by missense mutations in exons 5 through 9 in some cells and how this is detected by immunohistochemistry using commercial monoclonal antibodies. A specific example of detection of the cells that have this characteristic is given in Example 1. Applicants therefore submit that no new matter has been added by these amendments.

Claims 11, 18 and 19 are rejected as indefinite for recitation of the phrase "at least about." Claim 11 is amended herein to delete the word "about." Applicants therefore have overcome the rejection and request its withdrawal.

Claims 1, 5, 8-10 and 26 are rejected as anticipated by Jonason et al., based on the stated interpretation of the claim term "accumulated levels of p53" and in light of Brash. Jonason teaches identifying patches of cells with elevated p53 levels, not individual cells (see page 14025, paragraph 3; page 14026, paragraph 2), and amplifying DNA from these groups of cells. Individual, specific exons of the p53 gene (page 14026, paragraph 2 of "Results") were amplified separately after random amplification of the genome, a method that does not produce the PCR product of amended claim 1, which is an amplicon of at least 1 kb which spans exons 5-9. The Office may also note in this same paragraph that this method was hampered by "technical limitations" with respect to microdissection, which caused this method to produce obscured results which are avoided by the claimed method. The "random" amplification of the genome of the cell patches in no way discloses amplification of a DNA molecule at least 1 kb in size, since the methods of Jonason are amplification of random segments of indeterminate size throughout the genome, not amplification of a DNA spanning the entire chromosome or the entire genome.

Furthermore, there is no indication anywhere in Jonason that any amplicon of greater than 1 kb was ever created or that any attempt was made to amplify a DNA of that size, only of DNA in general. The Office has not pointed to anything in this reference concerning the size of the DNA sequences amplified other than the mere fact that the DNA is genomic DNA. Since amplification of so large an individual DNA segment spanning exons 5-9 of p53 was not disclosed or enabled by Jonason, Applicants submit that this claim limitation at least is missing

from the disclosures of Jonason. The reference also does not teach amplification of DNA from a single cell, providing a second, independent reason why the rejection of amended claim 1 is not proper over Jonason.

Claims 8-9 and 26 have been canceled, therefore the rejection is moot with respect to these claims. Applicants request that the rejection of claims 1, 5, 8-10 and 26 be withdrawn.

Claims 1, 5, 7-10 and 26 are rejected as anticipated by Diamandis, which discloses immuno-detection of p53 and amplification of individual p53-exons in a multiplex system to produce amplicons smaller than 1 kb and which do not span exons 5-9 of p53. See page 4, lines 13-24. The "immunoassays" of Diamandis cited by the Office involve detecting p53 in a sample of cells or patient serum, for example, Example 1 of Diamandis, pages 14-19, cited in the action is a test for anti-p53 antibodies in serum. The method does not identify a single somatic cell having accumulated p53.

The amplification of Diamandis is asserted by the Office to produce amplifications of "exon fragments" of 162-390 bases in length. Since the total length of these individual fragments adds up to more than 1kb, the Office concludes that a 1kb DNA has been amplified. It is clear that the Office is forced to concede that no amplification of a 1 kb or longer DNA molecule was performed, only several amplifications of a number of smaller DNAs. It is not the same achievement technically to amplify a group of small exon fragments in a group of cells versus to amplify a 1 kb DNA spanning several exons from a single cell. The fact that Diamandis amplified multiple small segments of

human genomic DNA is not relevant to amended claim 1, which requires amplification of a DNA molecule of at least 1 kb in size from a single human somatic cell.

Applicants submit that Diamandis does not teach or suggest that the claimed methods are even possible, much less provide enabling disclosure of all claim limitations. Because Diamandis does not disclose identifying a single somatic cell, or amplifying its DNA, or amplifying a 1kb DNA molecule to form a PCR product, or amplifying a DNA that spans exons 5-9, this reference does not anticipate claim 1. Applicants therefore request that the rejection of claims 1, 5, 7 and 10 be withdrawn. Claims 8-9 and 26 are canceled.

Claims 1, 2, 4 and 6 are rejected as anticipated by Miyajima. This reference relates to <u>non-fixed</u> frozen sections which were stained for p53 and considered positive if >10% of the cells stained. See p 178, section 2.2. The experimentors did not identify and microdissect a single somatic cell that stained positive for p53 accumulation and then amplify a DNA molecule of more than 1 kb from that single cell. Rather, they collected tumors and performed a single-stained conformation polymorphism analysis of the DNA fragments from groups of tumor cells. Table 1 shows that individual primer sets were used for individual exons of p53. See page 179. Therefore, it is clear that Miyajima did not disclose amplification of a 1 kb-long DNA molecule that spans exons 5-9, but only amplified smaller DNA segments corresponding to individual exons of p53. The primers for cDNA also included a primer at the junction between 2 exons or within one exon, so these primers also could not have amplified a DNA molecule that spans exons 5-9. Not only were

small DNAs amplified, but the amplifications were not performed on DNA from a single somatic cell identified as having p53 accumulation. This reference therefore also lacks several required claim elements.

Applicants therefore submit that the rejection of amended claim 1 is not proper given the complete lack of any teaching with respect to amplification of a 1 kb DNA or of amplification from a single somatic cell. Claims 2, 4 and 6 are canceled herein. Applicants request this rejection be withdrawn as to claim 1.

Claim 11 is rejected as obvious over Jonason and Leutenegger. Claim 11 is amended to depend from new claim 27. The complete lack of any teaching or suggestion in Jonason of amplification of a 1 kb DNA molecule from a single somatic cell identified as having p53 protein accumulated by missense mutations in exons 5 through 9 is discussed above. Leutenegger is cited for using calf thymus carrier DNA. This disclosure does not make up for the glaring deficiencies of Jonason et al. and does not relate to the limitation of claim 11, which relates to mouse carrier DNA rather than bovine. The Office does not cite any disclosure of mouse DNA or any carrier DNA having an average size of at least 20 kb. Applicants therefore submit that this rejection is improper; the references do not teach or suggest all limitations of claim 1 or claim 11. Applicants request this rejection be withdrawn.

Claims 12 and 20 are rejected as obvious over Jonason, Brash and Klein. Jonason lacks even the merest suggestion of amplifying DNA from a single somatic cell identified as having p53 accumulated by nussense mutations in exons 5-9 or of

amplifying a DNA molecule of 1 kb or longer from such a single somatic cell. The Office further admits that Jonason and Brash do not disclose or suggest single cells obtained by microdissection of paraffin-embedded tissue sections. Nor does Klein. Klein teaches that its methods may be used to amplify single-cell DNA from cryosections, but does not explain how this might be accomplished from a single cell from a paraffin-embedded tissue section, much less enable this.

In addition, Klein amplified a 1374 bp exon-containing fragment (exons 2-4) a 1032 bp exon-containing fragment (exons 5-6) and smaller fragments that contained exon 7 and exons 8-9. Klein did not amplify a DNA molecule that spans-exons 5-9. Even this limited work was not achieved in single cells from paraffin-embedded sections. The amplification certainly was not of a DNA molecule which is at least 1 kb in size and which spans exons 5 to 9 of p53, but rather of several DNA molecules that contain only some of the required exons. If the (not enabled) single-cell method of Klein were used in combination with the detection method of Jonason, the result still would not be amplification of a DNA molecule of at least 1 kb which spans exons 5-9 of p53. The combination would be a highly doubtful result, since the methods are not enabled for single paraffin-embedded single cells, and the amplification would be of small products not included in the present claim.

Therefore, even the combination of these references lacks teaching or suggestion of at least some claim elements since nothing in the cited art indicates one could amplify a DNA molecule that spans p53-exons 5-9 from a single cell, much less a

paraffin-embedded cell, regardless of the source. Applicants therefore request that this rejection be withdrawn.

Claim 13 is rejected as obvious over Jonason, Brash, Klein and Goldsworthy. The first three references, alone or in combination, completely lack teaching of amplification of a 1 kb or longer DNA molecule from a previously identified paraffin-embedded single somatic cell. Goldsworthy is cited for RNA amplification from single cells (fixed with ethanol, formalin, paraformaldehyde or acetone and embedded in paraffin) by RT-PCR. The methods of Goldsworthy are hardly comparable to the methods discussed above, at least in part because RNA is present in many more copies per cell than DNA, as is well known in the art. The motivation to combine these references as asserted by the Office is that ethanol fixation yielded the best morphology (which is not relevant to the methods claimed here), microdissection, and RNA extraction. RNA extraction is completely irrelevant to what is claimed and has no bearing on whether the DNA amplification methods of the claims would be successful. The two references therefore would not be combined with any expectation of success by the skilled person.

In any case, even if these two separate methods were combined, the most one would achieve is RNA extracted from a single cell, or if DNA were substituted even though there is no reason to believe DNA extraction would be achieved successfully with these methods, small DNA amplicons that would not span p53 exons 5-9. Applicants submit that this combination of references lacks a teaching or suggestion of all claim elements and also that there is no fair motivation to combine these teachings and then modify Goldsworthy to extract the much less plentiful DNA in

a single cell. The Office cannot make out a prima facie case of obviousness with respect to amended claim 1 and therefore should withdraw the rejection.

Claim 14 is rejected as obvious over Jonason, Brash, Klein and Aghassi. Klein's teachings are discussed above, in combination with Jonason, in light of Brash. These combined teachings are seriously deficient in not teaching or suggesting several claim elements as discussed above. The teaching of Aghassi with respect to steam heating in the presence of EDTA does not make up for these deficiencies and do not cause the Jonason methods to amplify a DNA molecule that spans exons 5-9 of p53. Since the Office cannot make out a prima facie case of obviousness using references which even in combination lack even the merest suggestion of multiple claim elements required in amended claim 1, as discussed above, this rejection should be withdrawn.

Claim 15 is rejected as obvious over Jonason, Brash and Klein. These references and their numerous deficiencies have been discussed at length above. The references do not teach or suggest any method for amplifying a DNA molecule that spans exons 5-9 of p53, even when combined. The Office again asserts that exons 4-9 were amplified in Klein, however, a careful reading clearly shows that only smaller fragments of DNA were amplified. Amplifying several small fragments is not the same as amplifying a large fragment that spans exons 5-9 of p53, particularly under the demanding technical conditions of single-cell amplification. Using one or two polymerases does not alter this basic fact. Applicants submit that this rejection is not proper against the

amended claims, which contain limitations completely lacking in the prior art and therefore should be withdrawn.

Claim 25 is rejected as obvious over Jonason, Brash, Murphy and Buck. Claim 25 has been amended to depend from new claim 27. Jonason does not teach a primer pair for amplifying a DNA spanning exons 4-9 of p53 as the Office asserts. These researchers amplified specific exons of p53 only, from groups of cells, and randomly amplified unidentified segments of the genome using "semi-random monamers." The Office asserts that Murphy teaches a primer which contains a sequence within it that is identical to SEQ ID NO:5, bases 5-28. Clearly this is not the same primer. Furthermore, primers operate in sets of two. This forward primer, as disclosed in Murphy, is non-operational alone. There is no reason for the skilled person to believe that it could be modified for use in the method which is claimed here, particularly since the references combined with Murphy do not disclose the appropriate modification or a reverse primer that could achieve the method, and since the references also lack disclosure of other required claim elements as discussed above.

Even if these references were combined and modified by the skilled person such that the Murphy primer were substituted for SEQ ID NO:5 in sequence analysis of the amplicon produced in claim 1, there still would have been no disclosure in the art that would produce an amplified DNA molecule spanning exons 5-9 of p53 to be analyzed. Applicants therefore request that this rejection be withdrawn, since being able to analyze a sequence which could not have been produced using prior art methods even when several references are combined does not make up for the

lack of required claim elements in the main references Jonason and Diamandis.

Claims 2, 3 and 6 are rejected as obvious over Diamandis and Hearslev. Claims 2, 3 and 6 have been canceled herein. Applicants therefore request this rejection be withdrawn.

Claim 11 is rejected as obvious over Diamandis and Leutenegger. Claim 11 has been amended to depend from new claim 27. The disclosures of each of the cited references have been discussed above. The Office asserts that the combination of amplification in the presence of the protein BSA and in the presence of calf thymus carrier DNA is basically equivalent to using mouse carrier DNA with an average size of at least 20 kb. The Office has cited no disclosure relating to mouse DNA or to any particular size of carrier DNA. The rejection therefore is improper. In addition, these types of disclosures regarding carriers do not make up for the deficiencies of the main reference, Diamandis, discussed above. Applicants therefore request the rejection be withdrawn.

Claim 12 is rejected as obvious over Diamandis, Hearslev and Klein. Hearslev is cited (in section 20) for teaching staining for p53 and PCNA, including paraffin-embedded cells. Klein is cited for amplifying exons 4-9 of p53 in single cells, however, as discussed above, Klein discloses amplification of several smaller DNAs, none of which span exons 4-9 (or 5-9) of p53. As discussed at length above, neither Diamandis nor Klein (nor Hearslev) disclose or suggest all claim limitations of claim 1 or claim 12. Hearslev does not teach or suggest the elements lacking in Diamandis or Klein, or when these references are

combined. Applicants therefore request that the rejection be withdrawn.

Claim 13 is rejected as obvious over the references cited against claim 12 above, with the addition of Goldsworthy. All these references have been discussed individually and in various combinations above. None disclose or suggest a method which can amplify a DNA molecule that spans exons 5-9 of p53 from a single paraffin-embedded cell. Combining references does not add disclosure that is not present in any of the references. Applicants submit that this rejection is not proper since no reference makes up for the deficiencies of the primary references discussed above. The Office should withdraw this rejection.

Claim 14 is rejected as obvious over the references cited against claim 12 above, with the addition of Aghassi in place of Goldsworthy. The addition of Aghassi does not make up for the deficiencies of the primary references any more than Goldsworthy. For the reasons discussed above, applicants request this rejection also be withdrawn.

Claim 15 is rejected as obvious over Diamandis and Klein. As discussed repeatedly above these references contain not even the merest hint as to several required claim limitations even if they were combined. Applicants refer the Office to discussions above concerning these references, both individually and in combination, and request the rejection be withdrawn.

Claim 18 is rejected over Diamandis, Leutenegger, Shamsher, Felix and Buck, none of which teach or suggest any method to amplify a DNA molecule that spans exon 5-9 of p53 from a single cell. Claim 18 is amended to depend from new claim 27. Diamandis is cited for teaching sequence analysis using SEQ ID

NOS:1-41 [sic], but not SEQ ID NOS:1 and 3. Claim 18 does not relate to SEQ ID NOS:1-41 or SEQ ID NOS:1 and 3 of the present application, but to SEQ ID NOS:1 and 2. Shamsher is cited for disclosing a sequence of 799 bases that includes SEQ ID NO:1. Felix is cited for a 133 bp insertion of intron 9 that includes SEQ ID NO:3 and amplification of introns. None of these sequences are identical to the primer pair of claim 18 or can achieve what this primer pair can. None of these sequences in the cited art references were even contemplated to amplify the DNA of claim 1, and there is no reasonable expectation that they could do so, even if they were cut and modified to produce one of the primer set of SEQ ID NO:1 and 2 (or 1 and 3) and then used in a method which is not even hinted at in the primary reference, as discussed at length above.

Further, the Office asserts that these primers necessarily will work in sequence analysis, according to Buck, on a "300 base pair" sequence. Office Action, page 25, line 14. Applicants submit that this is completely irrelevant to the claim presented here because these primers are not used to analyze a 300 base pair sequence, but to amplify a greater than 1 kb DNA molecule in a single somatic cell, which is not enabled or even suggested by Buck or by any of the other numerous cited references.

Applicants therefore submit that this rejection is not proper at least because (1) there is no disclosure of SEQ ID NO:2 in the cited references; (2) there is no motivation to modify the disclosed sequence to form SEQ ID NO:1; (3) there is no motivation to use SEQ ID NO:1 with SEQ ID NO:2; (4) none of the references teach or suggest the required claim elements of new claim 27, which are discussed above in the context of claim 1;

and (5) the reasoning behind the asserted usefulness of these primer sequences for sequence analysis of a 300 base sequence in Buck is not germane to the present claim. Applicants request that the rejection of this claim be withdrawn.

Claim 19 is rejected as obvious over Diamandis, Leutenegger, Shamsher, Accession No. X54156 and Buck. This claim also is amended to depend from new claim 27. As discussed in the paragraphs immediately above, these references do not disclose or suggest the primers of SEQ ID NOS: 1 and 3 (or SEQ ID NOS:1 and 2) which are claimed here, do not provide any motivation to combine these two primers and do not disclose several required claim limitations of claims 1 and 27 as discussed repeatedly herein. Moreover, the reasoning concerning the applicability of Buck is faulty as discussed above. Applicants request this rejection be withdrawn for reasons analogous to those cited above with respect to claim 18.

Claims 21-24 are rejected as obvious over Diamandis, Hearslev, Klein and Chang. These claims relate to the source of the sample and depend from claims 12 and 1. Applicants refer the Office to the discussions above with respect to the glaring deficiencies of the primary and secondary references above. Chang does not make up for these deficiencies because it also teaches or suggests nothing concerning how to amplify a DNA molecule spanning exons 5-9 of p53 from a single somatic cell in a paraffin-embedded tissue section. Applicants therefore request this rejection be withdrawn.

Claim 25 is rejected as obvious over Diamandis, Murphy and Buck. This claim has been amended to depend from new claim 27. Applicants refer the Office to the discussion with respect to the rejection of claim 25 over Jonason, Brash, Murphy and Buck, the

discussion of the inapplicability of Buck to the present claims, and to the lengthy discussion concerning the lack of teaching and suggestion in Diamandis. These combined references lack required claim elements of amended claim 1 and would not be combined with a reasonable expectation of success by the skilled person. Applicants therefore request that this rejection be withdrawn.

For the reasons discussed above, applicants request that all rejections be withdrawn at this time.

RESPECTFULLY SUBMITTED,					
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